



Cite this: *Anal. Methods*, 2021, 13, 34

Detection of RNA viruses from influenza and HIV to Ebola and SARS-CoV-2: a review

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RNA-based viruses likely make up the highest pandemic threat among all known pathogens in about the last 100 years, since the Spanish Flu of 1918 with 50 M deaths up to COVID-19. Nowadays, an efficient and affordable testing strategy for such viruses have become the paramount target for the fields of virology and bioanalytical chemistry. The detection of the viruses (influenza, hepatitis, HIV, Zika, SARS, Ebola, SARS-CoV-2, etc.) and human antibodies to these viruses is described and tabulated in terms of the reported methods of detection, time to results, accuracy and specificity, if they are reported. The review is focused, but not limited to publications in the last decade. Finally, the limits of detection for each representative publication are tabulated by detection methods and discussed. These methods include PCR, lateral flow immunoassays, LAMP-based methods, ELISA, electrochemical methods (e.g., amperometry, voltammetry), fluorescence spectroscopy, AFM, SPR and SERS spectroscopy, silver staining and CRISPR-Cas based methods, bio-barcode detection, and resonance light scattering. The review is likely to be interesting for various scientists, and particularly helpful with information for establishing interdisciplinary research.

Received 12th October 2020
Accepted 10th November 2020

DOI: 10.1039/d0ay01886d

rsc.li/methods

1. Introduction

Viruses are small, nanometer-scale carriers of genetic material. Unable to replicate by themselves, they live inside host cells and use their hosts for replication and assembly. Hence, they are intracellular parasites. In order to exist, viruses must be able to have multiple hosts. Viruses of bacteria, fungi, plants, and animals exist. Furthermore, a single virus can have hosts belonging to different species (e.g., rhinolophid bats and humans). Such viruses can jump from one host species to another. Viruses are not “interested” in killing their host instantaneously because the host’s death would lead to the termination of viral spread. Therefore, the majority of viruses do not cause lethality. Some viruses, however, are infectious agents capable of causing illnesses with lethal outcomes. According to the CDC, the flu caused 12 000–61 000 deaths in the USA each year since 2010.

Depending on the type of nucleic acid they carry, viruses are classified into DNA-containing and RNA-containing viruses. DNA-containing viruses can be single-stranded or double-stranded; they are typically more benign than RNA-viruses and mutagenize to a lesser degree. RNA-viruses can be positive-strand, negative-strand or ambisense. Positive-strand RNA viruses contain genomic RNA, which is identical to viral

mRNA, and can be translated by a host cell. Negative-strand viruses contain RNA in their genome, which is complimentary to mRNA and is used as a template for transcription by RNA-dependent RNA polymerase. Ambisense viruses contain genomic RNA, part of which behaves as positive-strand, while another part possesses negative-strand characteristics. For their replication, RNA-containing viruses rely on polymerases without stringent proofreading abilities. Contrarily, DNA-containing viruses rely on DNA-polymerase, which has proofreading properties. Therefore, the genome of RNA-containing viruses is subject to mutations at a much higher rate. This makes RNA-containing viruses rapidly evolving viruses that outnumber DNA-containing viruses. In addition, RNA-containing viruses are more pathogenic as compared to DNA-containing viruses. Examples of RNA-containing viruses that cause diseases include SARS-coronaviruses, influenza, hepatitis A, C, D, E, dengue, Ebola, HIV and other viruses.

Viruses are a diverse group of infectious agents, with more than 100 families characterized to date. Within the current review, we will focus on the laboratory diagnostics of RNA-containing viruses, which are causative agents of diseases with a high mortality rate or with a tendency for pandemic spread.

A multitude of different diagnostics tools exists. They include the detection of viruses, nucleic acids, and antibodies against viruses. The criteria that diagnostics tests should satisfy include a low limit of detection, high sensitivity, high specificity, high accuracy, and rapid speed of diagnosis. The limit of detection refers to the lowest concentration of analyte detected

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by a particular test. Depending on the test, the limit of detection can span a few RNA species in a reaction (for assays that detect nucleic acids) or a few ng mL⁻¹ of analyte (for assays that detect antibodies). The sensitivity refers to the ability of a particular test to detect a virus when the virus is present in a sample, and is expressed in % (100% – % of false negative results). Specificity refers to the ability of a particular test to show a negative result when the virus is absent from a sample, and is expressed in % (100% – % of false positive results). Accuracy denotes the percentage of times at which the performed test results are correct. Thus, a high accuracy indicates low percentages of false-positive and false-negative results. The time it takes for a particular test to produce results depends on the test. Antibodies-detecting tests take less time to produce results than nucleic-acid-based tests (such as quantitative real-time PCR). The present review is focused on comparing different viral diagnostic tests through a quantitative perspective.

Cheng *et al.*'s review in *Analytical and Bioanalytical Chemistry* gave a detailed picture of viral diagnostics in 2009,¹ where the detection was described using a method-to-method approach. Since the publication of that review, new lethal viruses have appeared (Ebola and SARS-coronaviruses), and many tests were developed and validated for their detection and diagnostics. The present review is focused on the quantitative analytical parameters in viral diagnostics as well, but it covers the subject from virus to virus. We selectively covered viruses that are lethal to humans, which contain RNA, and which have at least some pandemic potential. Most attention is directed towards the

COVID-19 pandemic virus. The papers described in this review typically reported the limit of detection or some other analytical parameters describing the test accuracy (selectivity, sensitivity) (Fig. 1). Moreover, the orthogonal approach of viral detection classified by detection method is briefly shown as a table at the end of the review.

2. Detection of influenza viruses

Influenza is a respiratory disease of viral origin. Two types of viruses, influenza A and influenza B, are causative agents² of the disease. Both viruses are negative-strand single-strand RNA viruses. The viral genome is segmented and contains 8 segments. They code for RNA-dependent RNA polymerase (needed to convert negative-strand into positive-strand RNA), haemagglutinin (HA, glycoprotein, required for viral entry), neuraminidase (NA, glycoprotein, needed for viral release), nucleoprotein (NP), matrix protein, membrane protein, nuclear export protein (NEP), and nonstructural proteins (NS). HA and NA proteins have high antigenic variability, contributing to the great diversity of viral subtypes. Influenza is characterized by seasonal epidemics, with an ability to transform into pandemics. The occurrence of pandemics is due to the zoonotic origin of the influenza A virus. It is of particular significance that the influenza A virus can spread among both animals and birds. The pandemics of 1918 and of 2009 (Spanish flu and swine flu, respectively) were caused by the H1N1 viral strain. The pandemic of 1918 resulted in more than 40 million deaths



Fig. 1 Graphic Content of the review: detection of RNA-containing pandemic-prone viruses by virus (top half) and by method of detection (bottom half).



worldwide. The 2009 swine flu pandemic was due to the H1N1 influenza A virus, and caused an estimated 201 200 respiratory deaths, together with 83 300 cardiovascular deaths; only 18 500 deaths were laboratory-confirmed.³ The WHO estimates that 290 000 to 650 000 deaths occur annually worldwide due to influenza-associated respiratory diseases.⁴ The CDC estimates that influenza has resulted in between 9 million and 45 million illnesses, between 140 000 and 810 000 hospitalizations, and between 12 000 and 61 000 deaths annually, since 2010 in the USA.⁵ The representative test for the detection of the H5N1 influenza virus is shown in Fig. 2, while different methods are presented in Table 1.

3. Detection of RNA-containing viruses of health concern

HIV/AIDS is prevalent mostly in East and Southern Africa, with a prevalence of 7.4% in the region in 2014, which is over 3 times higher than the prevalence in Western and Central Africa. Eastern Europe and Central Asia are the only regions with a rising HIV prevalence, at 0.8% in 2014. The global prevalence was 0.8% in 2014. The morbidity rate is currently at 1.1 million per year (2015).¹⁵ A variety of methods exist for HIV diagnostics. Two such methods are presented in Fig. 2 and 3. Both methods detect the p24 antigen by colloidal gold immunochromatography, and by ELISA with the use of AFM, respectively. In 2014, 10 000 people were infected with Ebola, with 4922 fatalities. The mortality rate for Ebola can reach 90%.¹⁶

In 2016, more than 85 countries and territories had the Zika virus infection transmitted by mosquitos. Brazil had the

greatest impact, with more than 200 000 cases of the Zika virus disease.¹⁷ The Zika virus had a mortality rate of 8.3% in Brazil.¹⁸

Cases of viral hemorrhagic fever were seen in Zimbabwe, Uganda, the Democratic Republic of Congo, Kenya, and Angola, with a fatality rate of up to 15%.¹⁹

The number of dengue cases reported to the WHO has increased over 15 fold over the last two decades, from 505 430 cases in 2000 to over 2 400 138 in 2010 and 3 312 040 in 2015. Deaths increased from 960 in 2000 to more than 4032 in 2015.²⁰

There are an estimated 1.4 million cases per year of hepatitis A, with 0.5% of mortalities due to viral hepatitis.²¹ Globally, an estimated 71 million people have chronic hepatitis C virus infection. The WHO estimated that approximately 399 000 people died from hepatitis C in 2016, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).²² Hepatitis D virus (HDV) globally affects nearly 5% of people who are chronically infected with the hepatitis B virus (HBV). The superinfection of HDV on chronic hepatitis B accelerates the progression to a more severe disease in all ages and in 70–90% of persons.²³ Every year, there are an estimated 20 million HEV infections worldwide. The WHO estimates that hepatitis E caused approximately 44 000 deaths in 2015 (accounting for 3.3% of the mortality due to viral hepatitis).²⁴ The summary of different methods to detect the outlined viruses of health concern is presented in Table 2.

4. Detection of coronaviruses

Coronaviruses are single-stranded, plus-strand, enveloped RNA-containing viruses, with a relatively large genome averaging at 30 kb pairs. Human coronaviruses (HCoV) 229E, NL63, OC43,



Fig. 2 SPR sensor for the detection of H5N1 avian influenza virus. The sensor is based on the changes of the refractive index of the plasmon as a result of binding the virion to the aptamer. The aptamer is bound to the gold surface of the sensor via biotin–streptavidin linkage. The portable sensor is capable of detecting 0.128 HAU, which is equal to $0.17 \times 10^{3.2}$ ELD₅₀ per mL. The image is reprinted with permission from H. Bai, R. Wang, B. Hargis, H. Lu and Y. Li, A SPR Aptasensor for Detection of Avian Influenza Virus H5N1, *Sensors*, 2012, 12(9), 12506–12518.



and HKU1 are known endemic human coronaviruses that cause mild respiratory infection with such symptoms as rhinorrhea and mild cough. Two other coronaviruses – Severe-Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses – are more virulent, and lead to a severe respiratory disease with symptoms similar to influenza. The SARS coronavirus caused a global epidemic in 2002–2003,^{85–87} killing 774 people out of the 8096 infected, and thus having a 9.56% mortality rate. MERS-CoV caused an epidemic in the Middle East that started in 2012.⁸⁸ By January 2020, MERS-CoV had killed 866 people out of the 2519 that were sick, and so it has a 34.4% death rate.⁸⁹

Diagnostic tests that use Polymerase Chain Reaction (PCR) allow for the detection of viral nucleic acid. With DNA-containing viruses, the PCR technique is more straightforward, as it depends on the amplification step of isolated viral DNA. The detection of RNA-containing viruses requires an additional step of converting RNA into DNA by reverse transcription. PCR utilizes synthetic single-stranded DNA primers and probes, and depends on the hybridization. Because primers and/or probes might bind nonspecifically to a region different from the intended one, PCR tests can result in false-positive outcomes. On the other hand, false-negative results might result from improperly collected material. The focus of the present review is to compare the key parameters of different test assays, such as specificity and selectivity, to get a quantitative outlook on the test systems.

There are different modalities of PCR, which are useful in viral detection. Among them are quantitative real-time PCR (qPCR), which allows for rapid detection with the identification



Fig. 3 Colloidal gold immunochromatographic assay to detect the recombinant p24 protein of HIV-1. The reported limit of detection for this method is 25 pg mL⁻¹. It is completed in 20 minutes with an accuracy of 98.03%. The image is reprinted with permission from Ma, Ni *et al.*, Development of Monoclonal Antibodies against HIV-1 p24 Protein and Its Application in Colloidal Gold Immunochromatographic Assay for HIV-1 Detection, *BioMed Research International*, 2016, 2016, 1–6.

of relative amounts of the nucleic acid. The quantitative real time RT-PCR (RT-qPCR) method is an efficient method of diagnostics, which allows for the rapid detection of viral nucleic acid. Many RT-qPCR methods are developed for the detection of coronaviruses. The range of methods for the detection of SARS and MERS coronaviruses is summarized in Table 3.

Table 1 Detection of influenza viruses

| # | Method | Target/analyte | Volume | Accuracy | Time for test | Ref. |
|---|---|--|------------------------------|--|---------------|------|
| 1 | Commercial antigen detection tests and RT-qPCR | Swine-origin influenza virus (S-OIV) and seasonal influenza A (H1N1) isolate: antigens and M genes | N/A | N/A | N/A | 6 |
| 2 | – Rapid antigen test – Direct immunofluorescence (DFA) – R-Mix culture – Respiratory Virus Panel (RVP) | H1N1 influenza A antigen | 200 µL of respiratory sample | Rapid antigen: 93.6% DFA: 94.5% R-Mix culture: 100% RVP: 100% | N/A | 7 |
| 3 | SPR aptasensor | Avian influenza virus H5N1 virions | N/A | N/A | 1.5 hours | 8 |
| 4 | ELISA | Anti-influenza A virus nucleoprotein antibodies | 15 µL of porcine sera | 99.3% Sensitivity – 96.6% | N/A | 9 |
| 5 | A double-antigen sandwich ELISA | Antibodies to influenza A viruses | 50 µL of serum sample | 97.3% Sensitivity – 98% | N/A | 10 |
| 6 | RT-LAMP on an integrated centrifugal disc | Influenza A (subtypes H1, H3, H5, H7, and H9) and influenza B RNA | 25 µL | N/A | 45 min | 11 |
| 7 | A nanocomposite of AuNPs and polyols with a dual response | Influenza A virions | N/A | N/A | N/A | 12 |
| 8 | Aptamer-based field-effect transistor | H5N1 avian influenza virus hemagglutinin (HA) protein | 3 µL | N/A | 5 hours | 13 |
| 9 | Magnetic particle spectroscopy | Influenza A virus subtype H1N1 nucleoprotein | 100 µL | N/A | 10 s | 14 |



Table 2 Detection of different RNA-containing viruses with health concern

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|---|---|------------------------------------|---|---------------------------------------|------|
| 1 | Colloidal gold immunochromatographic assay (GICA) | p24 (viral protein of HIV-1) | 75 µL r-p24 and 75 µL detector mAb | Specificity – 98.03% (1.96% – false-positive) | 20 min | 25 |
| 2 | Chemiluminescent magnetic microparticle-based immunoassay (ARCHITECT HIV Ag/Ab Combo) | HIV-1 p24 antigen | N/A | N/A | N/A | 26 |
| 3 | Fluorescent microparticle enzyme immunoassay (AxSYM HIV Ag/Ab Combo) | HIV p24 antigen | N/A | N/A | N/A | 26 |
| 4 | Enzyme-linked fluorescent assay (VIDAS HIV DUO Quick) | HIV p24 antigen | N/A | N/A | N/A | 26 |
| 5 | Enzyme-linked fluorescent assay (VIDAS HIV DUO Ultra) | HIV p24 antigen | N/A | N/A | 120 min | 26 |
| 6 | Quantitative enzyme-linked fluorescent immunoassay (VIDAS HIV p24 II) | HIV p24 antigen | N/A | N/A | N/A | 26 |
| 7 | Nanometer-scale antibody array-based analysis with AFM detection | HIV p24 antigen | 1 µL | N/A | 6 hours; 2–3 hours before measurement | 27 |
| 8 | Ultrasensitive capacitive immunosensor functionalized by anti-HIV-1 p24 mAb | HIV p24 antigen | 250 µL | N/A | 20 min | 28 |
| 9 | Boosted ELISA based on immune complex dissociation and amplified signal | HIV p24 antigen | 100 µL | N/A | >120 min | 29 |
| 10 | Nanoparticle-based biobarcode amplification assay | HIV p24 antigen | 100 µL | 100% | >120 min | 30 |
| 11 | Colorimetric lateral diffusion immunochromatography | Antibody against HIV | Finger prick blood | N/A | 3–30 min | 31 |
| 12 | Electrochemical ELISA | Antibody against HIV-1 and HIV-2 peptides | 20 µL | N/A | N/A | 32 |
| 13 | Electrochemical sensor, where antibodies bind to polypeptide epitopes | anti-HIV antibodies | 200 µL | N/A | 8 min | 33 |
| 14 | Reverse transcription loop-mediated isothermal amplification (RT-LAMP) products are visualized using a lateral flow immunoassay (LFIA): microfluidic rapid and autonomous analysis device (microRAAD) | HIV-1 RNA | 12 µL of human whole blood | N/A | 90 min | 34 |
| 15 | Luminescence assay | Ebola virus oligonucleotide (RNA) | N/A | N/A | 2 days | 35 |
| 16 | Reverse transcription loop-mediated isothermal amplification (RT-LAMP) | Ebola RNA | 1 µL of RNA | N/A | 1 hour | 36 |
| 17 | ELISA | Ebola virus nucleoprotein (NP) | 100 µL rNP | N/A | N/A | 37 |
| 18 | Fluorescence signal for quantitative detection and colorimetric signal for visual detection | Ebola virus glycoprotein | 50 µL | N/A | 20 min | 38 |



Table 2 (Contd.)

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|--|--|---|---|-------------------|------|
| 19 | Chemiluminescent ELISA | Antibodies against ebola virus strains Zaire and Sudan | 75 µL | Low cross-reactivity | N/A | 39 |
| 20 | Electroluminescent nanospheres and immunomagnetic separation | Ebola virions | 200 µL | N/A | 2 hours | 40 |
| 21 | Fluorescence assay on a micromagnetic platform | Ebola virions | 200 µL EBOV sample | Intra-assay CV = 4.9% | N/A | 41 |
| 22 | SERS immunoassay | Zika virions | 10 µL | N/A | N/A | 42 |
| 23 | Aptamer-based ELISA | Zika NS1 protein | 100 µL | N/A | N/A | 43 |
| 24 | Field effect biosensing | Zika viral antigen ZIKV NS1 | 75 µL | 1 : 10 dilution: CV = 19.89% 1 : 100 dilution: CV = 9.17% | N/A | 44 |
| 25 | Motion-based immunological detection | Zika virions | 10 µL of ZIKV | Correlation: 89.11% with the light microscopy; 100% with CDC Zika MAC-ELISA; 80% with Aptima Zika virus assay | >40 min for assay | 45 |
| 26 | Determination of isothermally Amplified Zika Virus RNA using a Universal DNA-Hairpin Probe | Zika Virus RNA | 50 µL | N/A | 1 hour | 46 |
| 27 | Advanced strand exchange amplification (ASEA) | Zika Virus RNA | N/A | N/A | 25 min | 47 |
| 28 | ELISA | anti-ZIKA IgM antibody | N/A | 87.5% positive agreement between CDC and InBios MAC-ELISAs | N/A | 48 |
| 29 | Single particle interferometric reflectance imaging sensor (SP-IRIS) cartridge | HFV virions (model – Ebola virions) | 100 µL | N/A | 20 min | 49 |
| 30 | Real-time reverse transcription-PCR | HFV RNA | 2 µL of RNA | No amplification of HIV-1, hepatitis B and C, herpes simplex type 1, cytomegalovirus, and Modoc viruses | >1 hour | 50 |
| 31 | qRT-PCR | HFV RNA | 5 µL of viral RNA | CV < 5%, no cross-reactivity | >1 hour | 51 |
| 32 | RT-LAMP | Crimean-Congo hemorrhagic fever (CCHF) RNA | 2 µL of the target RNA | 100% agreement between RT-LAMP and the nested PCR | 60 min | 52 |
| 33 | Fiber-optic biosensor with chemiluminescence | Crimean-Congo hemorrhagic fever (CCHF) IgG antibodies | 200 µL | N/A | 90 min | 53 |
| 34 | Loop-mediated isothermal amplification, LAMP | Dengue virus RNA | 2 µL of prepared RNA | The area under the ROC curve (AUC) = 0.95 | <1 hour | 54 |
| 35 | Biosensor with isothermal nucleic acid sequence-based amplification (NASBA) | Dengue virus RNA, serotypes 1, 2, 3, and 4 | 2 µL of amplicon (amplified dengue virus RNA) | Serotype 3 displayed low cross reactivity with biosensors designed for the detection of serotypes 1 and 4 | 15 min | 55 |
| 36 | Tandem toehold-mediated displacement reactions (tTMDR) with fluorescence | Dengue virus RNA | 100 µL | N/A | 35 min | 56 |
| 37 | Magnetic paper-based ELISA | Dengue immunoglobulin M (IgM) antibodies | 5 µL | N/A | N/A | 57 |
| 38 | Long-range surface plasmon polariton (LRSP) gold (Au) waveguides | Dengue IgM antibody | ~10 µL plasma | N/A | N/A | 58 |



Table 2 (Contd.)

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|---|--|--------------------------|--|---------------------|------|
| 39 | Stacking flow immunoassay | Dengue-specific immunoglobulin antibody | 100 µL | N/A | N/A | 59 |
| 40 | Separative extended gate field-effect transistor (SEGFET) as an immunosensor | Dengue virus nonstructural protein 1 (NS1) | N/A | N/A | <1 hour | 60 |
| 41 | Lateral flow immunoassay (LFIA) | Dengue NS1 protein | 10 mL | N/A | <1 hour | 61 |
| 42 | Magnetic separation and fluorescence detection | Dengue-2 virus virions | 20 mL | High specificity in the presence of yellow fever virus | 30–60 min | 62 |
| 43 | Electrochemical membrane-based nanobiosensor | Dengue-2 virus virions | 5 µL | RSD = 5.9% | N/A | 63 |
| 44 | RT-PCR | Hepatitis A virus (HAV) RNA | 5 µL of RNA extracts | N/A | >1 hour | 64 |
| 45 | qRT-PCR | HAV RNA | 5 µL of RNA extract | Regression coefficient of 0.9999 | >1 hour | 65 |
| 46 | Indirect competitive electrochemical immunosensor | HAV antigen | 100 µL | RSD < 3% | N/A | 66 |
| 47 | Electrochemical immunosensor | HAV antigen | 1.0 mL min ⁻¹ | RSD = 3.1–5.7% | 5 min | 67 |
| 48 | Solid-phase radioimmunoassay, HAVAB®-M | anti-HAV antibodies | 100 µL | RSD = 22% | N/A | 68 |
| 49 | Immunochromatographic assay (ICA) | anti-HAV IgM antibodies | 5 µL | Specificity = 100% | <20 min | 69 |
| 50 | Resonance light scattering (RLS) sensor | HAV virions | N/A | RSD = 1.3% | N/A | 70 |
| 51 | Multifunctional molecularly imprinted fluorescence sensor | HAV virions | 200 µL | RSD < 2.7% | 20 min | 71 |
| 52 | A reduced graphene oxide-assisted hybridization chain reaction + fluorescence | Hepatitis C virus (HCV) RNA | N/A | RSD = 3–6.4% | >8 hours | 72 |
| 53 | Capture of RNA with probes and paramagnetic particle separation | HCV RNA | 300 µL of serum | 93% sensitivity and 100% specificity | >1 hour | 73 |
| 54 | Electrochemical immunosensor | HCV antigen | 1.0 mL min ⁻¹ | RSD = 2.3–5.3% | 5 min | 67 |
| 55 | Chemiluminescent magnetic particle-based immunoassay | HCV core Antigen | A few hundred µL | 99% specificity; 97.4% sensitivity | 200 assays per hour | 74 |
| 56 | Sandwich electrochemical immunosensor | HCV core antigen | ≈ 10 µL | RSD = 3.1% | 30 min | 75 |
| 57 | Nano-gold immunological amplification and silver staining (NIASS) | anti-HCV antibodies | 10 µL | N/A | <40 min | 76 |
| 58 | Antibody-induced DNA strand displacement and rolling circle amplification (RCA) | anti-HCV antibodies | 1 µL | “High specificity” | 30 min | 77 |
| 59 | Immunogold electron microscopy | HCV virions | 3 µL | N/A | >3 hours | 78 |
| 60 | Dual-targeting real-time RT-PCR | Hepatitis D Virus (HDV) RNA | 140 µL viral sample | N/A | >1 hour | 79 |
| 61 | Real-time PCR | HDV RNA | 200 µL specimen | N/A | >20 min | 80 |
| 62 | Electrochemical immunosensor | HDV antigen | 1.0 mL min ⁻¹ | RSD = 3.4–6.8% | 5 min | 67 |



Table 2 (Contd.)

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|---|--|--------------------------|---|-----------|------|
| 63 | IgM capture enzyme immunoassay (EIA) | HDV antibodies IgM anti-HD | 100 µL | No cross-reactivity with other antibodies | 2 days | 81 |
| 64 | Real-time RT-PCR | Hepatitis E virus (HEV) subtype 3b RNA | 5 µL of extracted RNA | N/A | >1 hour | 82 |
| 65 | Array-based nano-amplification and silver stain enhancement | HEV RNA | 100 µL | N/A | 20–30 min | 83 |
| 66 | Electrochemical immunosensor | HEV antigen | 1.0 mL min ⁻¹ | RSD = 3.4–6.9% | 5 min | 67 |
| 67 | Enzyme immunoassay (EIA) | Antibody to the hepatitis E virus (anti-HEV) | 250 µL | N/A | N/A | 84 |

5. Detection of SARS-CoV-2 (COVID-19 pandemic virus)

On December 31, 2019, the WHO Chinese Country Office was notified about pneumonia cases of an unknown nature, epidemiologically linked to the seafood market in Wuhan, Hubei province.⁹⁷ At the same time, the Chinese CDC conducted an investigation in the field.⁹⁸ The viral nature of the disease was identified, 3 viral genomes from the bronchoalveolar lavage of three disease-affected individuals were sequenced, and the sequences were submitted to GISAID (accession ID: EPI_ISL_402119; EPI_ISL_402120; EPI_ISL_402121). According to

the sequencing data, the novel virus belongs to the *Coronaviridae* family, *Orthocoronaviridae* subfamily, *Betacoronavirus* genus, *Sarbecovirus* subgenus. The viral genome contains a 5' untranslated region (5'-UTR), replicase gene (orf1ab), Spike gene (S gene), Envelope gene (E gene), M gene, Nucleocapsid gene (N gene), and open reading frames 3, 7, 8, 10b, 13, and 14. The virus was named novel coronavirus 2019-nCoV, and the disease it causes was named novel coronavirus-infected pneumonia (NCIP) by the Chinese CDC.⁹⁹ Later, the WHO renamed the virus into SARS-CoV-2, and the disease into COVID-19.¹⁰⁰ The virus is highly contagious with easy person-to-person transmission, has a variable incubation period (from 4 to 24

Table 3 Detection of SARS and MERS coronaviruses

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|---|---|---|---|---|---|------|
| 1 | RT-PCR and indirect immunofluorescence serologic testing | SARS-CoV RNA | 2 mL nasopharyngeal aspirates and 2 g feces | RT-PCR: 60% for positive cases; 99.4% for negative cases Serologic testing: 92% for positive cases; 92% for negative cases | RT-PCR: N/A Serologic testing: >1 hour | 90 |
| 2 | Real time RT-PCR (2 assays: for upE and (ORF)1b) | SARS-CoV RNA: upstream of the E gene (upE) or within open reading frame (ORF)1b | 5 µL of extracted RNA | 100% specificity for both for upE and (ORF)1b | 36+ hours | 91 |
| 3 | Chemiluminescence immunosorbent assay with nanoarray RNA aptamer | SARS-CoV nucleocapsid protein (SARS-CoV N protein) | N/A | C-terminal domain or dimer form N protein is specifically recognized by the aptamer | N/A | 92 |
| 4 | Biosensor assay based on an optical QDs-based RNA aptamer | SARS-CoV nucleocapsid protein (SARS-CoV N protein) | N/A | QDs-conjugated RNA aptamer is selective against the SARS-CoV N protein | 1 hour | 93 |
| 5 | Asymmetric five-primer reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay | MERS-CoV RNA (3 genetic loci: ORF1a, ORF1b and E) | 4 µL RNA | N/A | 30–50 minutes | 94 |
| 6 | ELISA | MERS-CoV nucleocapsid protein (NP) | 50 µL of sample | 100% specificity | N/A | 95 |
| 7 | ELISA and plaque-reduction neutralization test (PRNT) | Antibodies against MERS-CoV | N/A | No serotypic discrimination between the MERS-CoV strains | N/A | 96 |



Table 4 Detection of SARS-CoV-2

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|---|--|-------------------------------------|--|---|-------------------|
| 1 | Real time RT-PCR Commercial kit from Altona diagnostics, Hamburg, Germany | E-gene RNA and S-gene RNA | 30 µL | N/A | >1 hour | 111 and 112 |
| 2 | ePlex-based (DNA hybridization and electrochemical detection) SARS CoV-2 | cDNA coding for nucleocapsid (N) | 200 µL of nasopharyngeal swab | Detection of positive – 94.4%; 95% CI – 74.2– 99% Detection of negative – 100%; 95% CI – 92.4– 100% | >1 hour | 111 and 113 |
| 3 | Real-time RT-PCR | RNA coding for RdRp gene | N/A | N/A | >1 hour | 114 |
| 4 | Real-time RT-PCR | SARS-CoV-2 RNA coding for nucleocapsid gene (N1 probe) | 20 µL | N/A | >1 hour | 111 and 114 |
| 5 | Real-time RT-PCR | SARS-CoV-2 RNA coding for nucleocapsid gene (N2 probe) | 20 µL | N/A | >1 hour | 114 |
| 6 | Real-time RT-PCR (E-gene assay, and RdRp gene assay) | SARS-CoV-2 RNA coding for envelope (E) gene and RNA- dependent RNA polymerase (RdRp) gene | 25 µL | No reactivity on human coronaviruses | >1 hour | 105 |
| 7 | Real-time RT-PCR | Co-V2 viral RNA; probe against nucleocapsid gene (N and N2 assays) | N/A | N/A | >1 hour | 115 |
| 8 | Real-time RT-PCR | SARS-CoV-2 RNA | 5 µL RNA | No cross-reactivity with other human- pathogenic coronaviruses and respiratory pathogens | >1 hour | 116 |
| 9 | RT-PCR: Cepheid Xpert Xpress and Roche cobas assays | SARS-CoV-2 RNA | N/A | Both systems have agreement of 99% | 45 min (Cepheid); 90 min (Roche) | 117 |
| 10 | RT-LAMP (reverse transcription loop- mediated isothermal amplification assay) | Viral RNA coding orf1ab gene and S gene | 25 µL | Sensitivity – 100% (95% CI 92.3–100%) Specificity – 100% (95% CI 93.7–100%) | 26.28 ± 4.48 min | 107 |
| 11 | Colorimetric LAMP | SARS-CoV-2 RNA | 3 µL RNA | 100% agreement with RT-PCR | 30 min | 118 |
| 12 | RT-LAMP | Viral RNA coding for conserved region within nucleocapsid gene | N/A | Sensitivity – 100% Specificity – 98.7% | 30 min (colorimetric visualization) | 108 |
| 13 | RT-LAMP | Viral RNA within RdRp gene | 25 µL | 100% consistency with RT-qPCR on positive samples | 50 min real-time monitoring | 109 |
| 14 | DETECTR (SARS-CoV-2 DNA endonuclease- targeted CRISPR trans reporter) – CRISPR-Cas12- based assay | Viral RNA coding for nucleoprotein and envelope genes | N/A | 95% for positive samples, 100% for negative samples | 30–40 min (sample-to- result) | 110 |
| 15 | ELISA | SARS-CoV-2 neutralizing, spike- and nucleocapsid- specific antibodies | N/A | 87–100% specificity | 2 days | 119 |
| 16 | COVID-19 IgG/IgM rapid test Cassette | SARS-CoV-2-specific IgM and IgG | 5 µL serum | Specificity 100% for IgM and 99.2% for IgG Sensitivity 69% for IgM and 93.1% for IgG | 15 min | 120 |
| 17 | Colloidal gold Antibodies test | SARS-CoV-2-specific IgM and IgG | 10 µL of serum | Specificity: IgM, 50.0%; IgG, 87.5% | N/A | 121 |
| 18 | Single molecule array (Simoa) immunoassay | SARS-CoV-2 nucleocapsid protein (N-protein) | 20 µL of whole blood | 100% specificity and 97.4% sensitivity | N/A | 122 |
| 19 | Pulse-controlled amplification (PCA) | SARS-CoV-2 RNA (E gene) | 45 µL | 100% agreement with RT-qPCR | 20 min | 123 |
| 20 | LAMP | SARS-CoV-2 RNA | N/A | N/A | <30 min | 124 |



Table 4 (Contd.)

| # | Method summary | Analyte | Sample volume | Accuracy | Time | Ref. |
|----|--|----------------|--|----------------------------|----------|------|
| 21 | Reverse-transcription recombinase-aided amplification (RT-RAA) | SARS-CoV-2 RNA | 140 µL nasopharyngeal swabs and sputum | 100% agreement with RT-PCR | 5–15 min | 125 |

Table 5 Detection of RNA-containing lethal viruses: classification by method of detection

| PCR-based nucleic acid detection | | | | |
|--|-----------|---|-----------|---|
| Analyte | Ref. | Analytical parameters | Ref. | Analytical parameters |
| Viral hemorrhagic fever (HF) viruses: Ebola, Marburg, Lassa, Crimean-Congo HF, Rift Valley fever, dengue, yellow fever viruses | 50, 2002 | LOD = 1545 to 2835 viral genome equivalents per mL of serum (8.6 to 16 RNA copies per assay) | 51, 2014 | LOD = between 45 and 150 cRNA/rxn |
| Hepatitis A | 64, 2009 | LOD = 1 PFU/1.5 L | 65, 2010 | LOD = 10 PFU/1.5 L of bottled water, 100 PFU/1.5 L of tap water |
| Hepatitis C | 73, 2000 | LOD = 33 cRNA per mL; ($\sim 1.74 \times 10^{-4}$ pg mL ⁻¹) | | |
| Hepatitis D | 79, 2018 | LOD = 575 IU mL ⁻¹ | 80, 2013 | LOD = 7500 HDV cRNA per mL; 190 cRNA/rxn (~ 0.28 pg mL ⁻¹) |
| Hepatitis E | 82, 2013 | LOD = 25 UI mL ⁻¹ | | |
| Seasonal influenza A | 6, 2009 | LOD = log ₁₀ 6.5–7.1 of M gene copies | | |
| Swine-origin influenza A | 6, 2009 | LOD = log ₁₀ 6.5–7.3 of M gene copies | | |
| SARS-CoV | 90, 2004 | LOD = 10 cRNA/rxn; Ac = 60% for positive cases, 99.4% for negative cases | 91, 2012 | LOD = 3.4 cRNA/rxn for upstream of the E gene (upE) ($\sim 1.11 \times 10^{-2}$ pg mL ⁻¹) and 64 cRNA/rxn for within open reading frame (ORF)1b, Sp = 100% |
| SARS-CoV-2 | 116, 2020 | LOD = 11.2 cRNA/rxn ($\sim 3.67 \times 10^{-2}$ pg mL ⁻¹) | 111, 2020 | LOD = 24 cRNA/rxn ($\sim 1.97 \times 10^{-2}$ pg mL ⁻¹) |
| | 105, 2020 | LOD = 3.8 cRNA/rxn ($\sim 2.49 \times 10^{-3}$ pg mL ⁻¹) – RdRp-gene assay; 5.2 cRNA/rxn ($\sim 3.41 \times 10^{-3}$ pg mL ⁻¹) – E-gene assay | 115, 2020 | LOD = 25 and 250 cRNA/rxn |
| Lateral flow immunoassays | | | | |
| HIV-1 p24 antigen | 25, 2016 | LOD = 25 pg mL ⁻¹ | | |
| Ab against HIV | 31, 2006 | LOD = sub pmol L ⁻¹ range | | |
| HIV-1 RNA | 34, 2019 | LOD = 3×10^5 HIV-1 viral particles, or 2.3×10^7 virus copies per mL of whole blood | | |
| Dengue NS1 protein | 61, 2020 | LOD = 5 ng mL ⁻¹ | | |
| ELISA | | | | |
| HIV p24 antigen | 29, 2003 | LOD = 0.5 pg mL ⁻¹ | | |
| Ab to HIV-1, HIV-2 | 32, 2013 | LOD = 1 ng mL ⁻¹ (6.7 pM) | | |
| Ebola virus nucleoprotein (NP) | 37, 2001 | LOD = 30 ng of purified recombinant NP (rNP) ($\sim 3.00 \times 10^5$ pg mL ⁻¹) | | |
| Zika NS1 protein | 43, 2017 | LOD = 0.1 ng mL ⁻¹ | | |
| IgM-dengue antibodies | 57, 2017 | LOD = 0.04 µg mL ⁻¹ | | |
| MERS-CoV nucleocapsid protein (NP) | 95, 2015 | LOD = 10 TCID ₅₀ /0.1 mL | | |



Table 5 (Contd.)

| PCR-based nucleic acid detection | | | | |
|---|----------|--|----------|---|
| Analyte | Ref. | Analytical parameters | Ref. | Analytical parameters |
| AFM | | | | |
| HIV p24 antigen | 27, 2004 | LOD = 25 fg mL ⁻¹ | | |
| Electrochemical detection | | | | |
| HIV p24 antigen | 28, 2010 | LOD = 7.9 × 10 ⁻⁸ pg mL ⁻¹ | | |
| Anti-HIV antibodies | 33, 2012 | LOD = 1–10 nM (~1.50 × 10 ⁵ pg mL ⁻¹) | | |
| Ebola virions | 40, 2017 | LOD = 5.2 pg mL ⁻¹ | | |
| Zika viral antigen ZIKV NS1 | 44, 2018 | LOD = 450 pM | | |
| Zika Virus RNA | 46, 2019 | LOD = 1.11 fg μL ⁻¹ (~0.3 fM) | | |
| Dengue virus nonstructural protein 1 (NS1) | 60, 2014 | LOD = 0.25 μg mL ⁻¹ | | |
| Dengue-2 virions | 63, 2012 | LOD = 1 PFU mL ⁻¹ | | |
| H5N1 avian influenza virus hemagglutinin (HA) protein | 13, 2020 | LOD = 5.9 pM | | |
| Hepatitis A antigen | 66, 2017 | LOD = 26 × 10 ⁻⁵ IU/mL | 67, 2010 | LOD = 0.5 ng mL ⁻¹ |
| Hepatitis C antigen | 67, 2010 | LOD = 0.8 ng mL ⁻¹ | 75, 2017 | LOD = 3 fg mL ⁻¹ |
| Hepatitis D antigen | 67, 2010 | LOD = 0.5 ng mL ⁻¹ | | |
| Hepatitis E antigen | 67, 2010 | LOD = 1 ng mL ⁻¹ | | |
| Chemiluminescence detection | | | | |
| HIV p24 antigen | 26, 2011 | LOD = 18–25 pg mL ⁻¹ or 1.24 IU mL ⁻¹ | | |
| Hepatitis C Virus (HCV) core antigen | 74, 2006 | LOD = viral concentration equivalent to the lowest titer of 2415 cRNA/mL | | |
| Fluorescence detection | | | | |
| HIV p24 antigen | 26, 2011 | LOD = 22–77.4 pg mL ⁻¹ or 1.94–2.25 IU mL ⁻¹ | 26, 2011 | LOD = 13 pg mL ⁻¹ or 0.43 IU mL ⁻¹ |
| | 26, 2011 | LOD = 11.5–25 pg mL ⁻¹ or 0.66 IU mL ⁻¹ | 26, 2011 | LOD = 11.2 pg mL ⁻¹ or 0.73–1.15 IU mL ⁻¹ |
| Ebola RNA | 35, 2016 | LOD = femtomolar level | | |
| Ebola glycoprotein | 38, 2017 | LOD = 0.18 ng mL ⁻¹ | | |
| Ebola virions | 41, 2018 | LOD = 2.6 pg mL ⁻¹ | | |
| Dengue virus RNA | 56, 2018 | LOD = 6 cRNA per sample (~3.62 × 10 ⁻⁴ pg mL ⁻¹) | | |
| Dengue-2 virions | 62, 2008 | LOD = 10 PFU mL ⁻¹ | | |
| HAV virions | 71, 2019 | LOD = 3.4 pmol L ⁻¹ | | |
| HCV RNA | 72, 2019 | LOD = 10 fM | | |
| anti-HCV Ab | 77, 2019 | LOD = 0.998 pM | | |
| SARS-CoV nucleocapsid protein (SARS-CoV N protein) | 92, 2009 | LOD = 2 pg mL ⁻¹ | | |
| LAMP-based nucleic acid detection | | | | |
| Ebola | 36, 2017 | LOD = 100 cRNA (~1.04 pg mL ⁻¹) | | |
| Crimean-Congo hemorrhagic fever | 52, 2013 | LOD = 0.1 fg of viral RNA (equivalent to 50 viral particles; ~0.05 pg mL ⁻¹) | | |
| Dengue | 54, 2020 | LOD = 10 ² PFU per 200 μL of whole blood | | |
| Influenza A | 11, 2020 | LOD of subtypes H1: 50 copies H3: 20–50 copies H5: 50 copies H7: 20–50 copies H9: 50–100 copies | | |



Table 5 (Contd.)

| PCR-based nucleic acid detection | | | | |
|--|-------------------|--|-----------|---|
| Analyte | Ref. | Analytical parameters | Ref. | Analytical parameters |
| Influenza B | 11, 2020 | LOD = 50 copies | | |
| MERS-CoV | 94, 2015 | LOD = 0.02 to 0.2 PFU (5 to 50 PFU mL ⁻¹) | | |
| SARS-CoV-2 | 118, 2020 | LOD = 120 cRNA/rxn (or 4.8 copies per μ L); $\sim 7.87 \times 10^{-2}$ pg mL ⁻¹ | 107, 2020 | LOD = 20 cRNA/rxn – ORF1ab gene ($\sim 1.31 \times 10^{-2}$ pg mL ⁻¹) and 200 cRNA/rxn – S gene (~ 0.131 pg mL ⁻¹) |
| | 109, 2020 | LOD = 3 cRNA/rxn ($\sim 1.97 \times 10^{-3}$ pg mL ⁻¹) | | |
| CRISPR-Cas based | | | | |
| SARS-CoV-2 | 110 and 128, 2020 | LOD = 10 cRNA/rxn (~ 0.164 pg mL ⁻¹) | | |
| Biobarcode detection | | | | |
| HIV p24 antigen | 30, 2007 | LOD = 0.1 pg mL; Ac = 100% | | |
| Motion-based detection | | | | |
| Zika virions | 45, 2018 | LOD = 1 particle/ μ L | | |
| NASBA-based nucleic acid detection | | | | |
| Zika | 46, 2019 | LOD = 1.11 fg μ L ⁻¹ (~ 0.3 fM) | | |
| Dengue | 55, 2002 | LOD = 10 PFU mL ⁻¹ | | |
| Advanced strand exchange amplification-based nucleic acid detection | | | | |
| Zika | 47, 2018 | LOD = 1.0×10^{-15} M; (~ 33 pg mL ⁻¹) | | |
| Interferometric reflectance imaging | | | | |
| HFV virions | 49, 2017 | LOD = 10 viruses per spot | | |
| Surface plasmon polariton waveguides | | | | |
| Dengue-specific immunoglobulin M (IgM) antibody | 58, 2014 | LOD = ~ 22 pg mm ⁻² | | |
| SERS (Surface Enhanced Raman Spectroscopy) | | | | |
| Zika virions | 42, 2018 | LOD = 10 ng mL ⁻¹ | | |
| FCV virions | 129, 2005 | LOD = 10^6 viruses per mL | | |
| Radioimmunoassay | | | | |
| Anti-HAV Ab | 68, 1993 | LOD = 10 mIU mL ⁻¹ | | |
| Resonance light scattering | | | | |
| HAV virions | 70, 2017 | LOD = 8.6 pmol L ⁻¹ | | |
| Silver staining | | | | |
| HCV antibodies | 76, 2005 | LOD = 3 ng mL ⁻¹ | | |
| HEV RNA | 83, 2006 | LOD = 100 fM; (~ 237.6 pg mL ⁻¹) | | |
| Electron microscopy | | | | |
| HCV virions | 78, 2006 | LOD = 10^7 virions per mL | | |
| SPR | | | | |
| AIV H5N1 virions | 8, 2012 | LOD = 0.128 HAU | | |
| Confocal laser scanning microscopy | | | | |
| SARS-CoV nucleocapsid protein | 93, 2011 | LOD = 0.1 pg mL ⁻¹ | | |



Table 5 (Contd.)

| PCR-based nucleic acid detection | | | | |
|---|----------|--|------|-----------------------|
| Analyte | Ref. | Analytical parameters | Ref. | Analytical parameters |
| Magnetic particle spectroscopy | | | | |
| H1N1 nucleoprotein molecule | 14, 2020 | LOD = 4.4 pmoles | | |
| Nanocomposite-based optical and mechanical detection | | | | |
| Influenza A virions | 12, 2020 | LOD = 5×10^7 PFU mL ⁻¹ | | |

days),¹⁰¹ and leads to the development of respiratory diseases with variable symptoms, from a mild cough to pneumonia.¹⁰² Sometimes, the infected person does not show any symptoms whatsoever.¹⁰³ Soon after the disease outbreak, close monitoring of the epidemiological situation around the globe started with a real-time count of new cases in the world.¹⁰⁴ Strict quarantine measures were implemented in the countries most affected by the disease, and on March 11th, the WHO declared a state of pandemic. Accurately monitoring the virus and its spread is not possible without reliable diagnostic tools. So, soon after the outbreak, the development of tests detecting SARS-CoV-2 itself or the immune response in the affected person was initiated.

A sample for the test includes material from the upper and lower respiratory tracts, and can include aspirates, oropharyngeal and nasopharyngeal swabs, bronchoalveolar lavage, and sputum, as well as nasal and nasopharyngeal aspirate. Test results depend on the quality of the collected material, as well as on the type of material.

Multiple assays were developed to test the presence of viral nucleic acid. The tests for SARS-CoV-2 detection employ a probe to detect the sequence within the RNA-dependent RNA polymerase viral gene (RdRp), as well as the nucleocapsid gene (NP), envelope protein gene (E), and spike protein gene (S). Some tests rely on the usage of fluorescently labeled TaqMan probes with a fluorescent reporter and a quencher attached to the 5'- and 3'-ends of the probe, respectively. Examples of such probes include probes with 6-carboxyfluorescein (FAM) or HEX dye at their 5'-end, and with a blackberry quencher (BBQ) or blackhole quencher (BHQ) at the probe's 3'-end.¹⁰⁵ The 5'-exonuclease ability of DNA-polymerase removes the fluorescently-labeled 5'-end of the hybridized probe, which leads to probe degradation and unquenching of the fluorescent reporter. There are 14 probes provided by the WHO, and one described by Zhu *et al.*⁹⁸ Most of them use TaqMan probes, with one exception from Japan that relies on nested PCR instead. Information about the limit of detection and the accuracy of the real-time reverse-transcription tests is gathered in Table 4.

Alongside real-time RT-PCR, isothermal hybridization is a method of choice for viral RNA detection in a shorter period of time, as compared to real-time RT-PCR. Originally invented by Notomi,¹⁰⁶ the so-called LAMP (loop-mediated isothermal amplification) coupled to reverse transcription is also used for SARS-CoV-2 detection,^{107–109} and allows for the rapid detection of viral

RNA within a time frame of less than 1 hour. The method relies on the strand-displacing ability of the Bst polymerase, and needs 4 to 6 primers, which increases the target selectivity of this method, as compared to regular PCR with only 2 primers.¹⁰⁶ The method can be run on a regular thermostat at 65 °C. The sensitivity of the RT-LAMP method is slightly lower (~10-fold) than that of real-time RT-PCR^{108,109} for SARS-CoV-2 detection.

The most novel method of viral detection, and possibly the most intriguing one, uses CRISPR-Cas 12 endonuclease and isothermal amplification.¹¹⁰ This method, named DETECTR, is very rapid, allowing for the detection of the viral RNA within minutes. It is a very promising point-of-care test that does not require expensive equipment and can be used in developing countries.

6. Summary of detection methods

Nucleic acid detection methods including PCR and LAMP

Comparing the different methods of viral detection, PCR-based methods can detect a few copies of RNA per reaction, which places them on the high-sensitivity spectrum, corresponding to a fg mL⁻¹ concentration range. PCR detection sensitivity is comparable between different viruses, with a range of detection from a few copies per reaction to a few hundreds of copies per reaction. PCR-based methods require trained personnel and expensive equipment, such as the PCR thermocycler. They are also time-consuming and can take several hours. In order to facilitate PCR-based techniques without compromising selectivity and specificity, alternatives to RT-qPCR exist. These include the LAMP technique. LAMP has a limit of detection comparable to that of RT-PCR, spanning from 3 copies of RNA per reaction to 100 copies of RNA per reaction, as evidenced from 5 sources presented in Table 5.

LAMP-based diagnostic tests allow for the rapid detection of the analyte, without a need for expensive equipment. Results can be obtained rapidly (within an hour), which makes these tests a suitable platform to be used for the development of point-of-care diagnostics tests. Alongside LAMP, the NASBA-based nucleic acid detection,^{46,55} as well as the advanced strand exchange amplification-based nucleic acid detection, are used and demonstrate a low limit of detection. A standard PCR test can also be incorporated into the assay, with other methods of separation such as laser-irradiated DNA extraction, paramagnetic particle separation, and others. Today, stationary PCR-based methods requiring expensive and complicated



equipment are accompanied by bench scale PCR detection with portable devices and automated procedure, where only one step of sample loading is necessary, such as with the “Cepheid Xpert Xpress” and “Roche cobas” assays.¹¹⁷

Immunoassay-based methods

The average limit of detection for ELISA-based methods is within the pg mL^{-1} range, but has been demonstrated to be as low as a fraction of pg mL^{-1} .²⁹ ELISA-based methods are routinely used and have demonstrated their applicability for an extended period. Despite this, they require trained personnel and are time-consuming. Therefore, alternative methods are being developed to make diagnostics tests relatively cheap and affordable without compromising their sensitivity and selectivity. One of these methods is lateral flow immunoassay. This method has shown a limit of detection at the pg mL^{-1} level, which is comparable to fine ELISA-based methods, but lateral flow immunoassays are faster. For example, a standard ELISA can take anywhere from 2 hours²⁹ to 2 days,¹¹⁹ as studies show. However, lateral flow immunoassays take from 20 minutes⁴⁷ to 90 minutes,³⁴ on average. This makes lateral flow immunoassays a good alternative to ELISA, especially because they can be performed at a small scale. The selectivity of immunoassay-based methods allows for the distinguishing of viruses with 100% specificity,⁹⁵ but not for making distinctions between different strains of the same virus.⁹⁶ Like PCR-based methods, immunoassay-based methods are now commercially available in bench scale, portable, fully automated devices, suitable for point-of-care testing.¹²⁰ However, these are currently qualitative, and their sensitivity is unknown. Immunoassay-based methods can detect a viral antigen or antibodies to the virus in the sample. The latter is more widespread for diagnostic testing, but can only be conclusive after the onset of symptoms (on average, a week after infection).

Electrochemical detection methods

Electrochemical detection methods provide sensitive detection of a wide range of analytes: virions,⁴⁰ viral antigen,⁴⁴ antibodies to the virus,³³ and viral nucleic acids.⁴⁶ The main advantage of these detection methods is that they are relatively inexpensive and not limited by a diffraction limit (like optical methods). The lowest limit of detection was reported by Teeparuksapun *et al.*²⁸ to be at a subattogram per milliliter concentration. Other studies show a pg mL^{-1} to fg mL^{-1} limit of detection range, which is comparable to PCR and immunoassays. The specificity of these methods is also high.³³ Notably, these detection methods also provide reproducible results with $\text{RSD} < 5\%$, such as the voltammetry method of Tang *et al.*,⁶⁷ which allows the multiplex detection of five analytes (viral antigens). Multiplex analyte detection has been reported in many research papers, which gives electrochemical methods an advantage over PCR and immunoassays. Electrochemical methods can be tuned to perform wash-free analysis³³ as well. Electrochemical detection methods usually use one of three approaches: voltammetry, amperometry, and detection of the change in capacitance. All three can be used in fast analysis,^{28,33,67} as the reported time of

experiments ranged between 5 and 20 minutes. This is much faster than the majority of PCR tests and immunoassays. However, these electrochemical methods require complicated equipment (for example, the use of gold nanoparticles with TEM and AFM imaging), highly trained operators, solution media, and are not suitable for point-of-care testing. Currently, the potential use of these methods in diagnostic testing is improbable because they require the fabrication of electrochemical sensors, often on a nanoscale level.

Fluorescence

Fluorescence spectroscopy is a well-established technique of sensitive detection. Its limit of detection is in the pg mL^{-1} range, on average, as can be seen in Table 5. Fluorescence signal detection can be incorporated in other procedures, such as immunoassays for the detection of antibodies or antigens.²⁶ Immunoassays with fluorescence signals have already been commercialized, and are even available in portable devices for home and point-of-care testing.²⁶ Fluorescence is reported as a reproducible method with $\text{RSD} < 5\%$.⁴¹ Other fluorescence-based methods use nanospheres, micromagnetic platforms, or upconversion materials for creating luminescence, which is further absorbed by nanoparticles conjugated to the target analyte. This method allows for more sensitive detection at the femtomolar level.³⁵ However, it is complicated and requires the fabrication of assay components, as well as highly trained personnel. Fluorescence signal acquisition is fast (around 20 minutes, on average), but the whole assay would take much longer to complete if the assay components need to be fabricated for fluorescence-based detection. Fluorescence-based immunoassays are faster (the whole assay takes around 2 hours), but their limit of detection is higher.²⁶ Fluorescence is



Fig. 4 AFM-based three-component ELISA using dip-pen nanolithography for HIV p24 antigen detection. The current method allows for detection of 25 fg mL^{-1} , which corresponds to 50 RNA copies per mL. The method shows at least 200-fold higher sensitivity compared to the 5 pg mL^{-1} limit of detection of conventional ELISA. The image is reprinted with permission from K. B. Lee, *et al.* The Use of Nanoarrays for Highly Sensitive and Selective Detection of Human Immunodeficiency Virus Type 1 in Plasma, *Nano Letters*, 2004, 4(10), 1869–1872. Copyright 2004 American Chemical Society.





Fig. 5 E-DNA antibody sensor. The sensor (top) comprises an electrode-bound, redox-reporter-modified DNA strand, termed the "anchor strand," that forms a duplex with a complementary "recognition strand" (here composed of PNA) to which the relevant recognition element is covalently attached. In the absence of antibody binding (top middle), the flexibility of the surface attachment chemistry supports relatively efficient electron transfer between the redox reporter and the electrode surface. Binding to the relevant target antibody (top right) decreases electron transfer, presumably by reducing the efficiency with which the reporter collides with the electrode. Binding can thus be measured as a decrease in the peak current as observed *via* square wave voltammetry (bottom). As shown, sensors in this class are highly selective and perform equally well in buffered saline (bottom middle), undiluted blood serum (bottom right), or 1 : 4 diluted whole blood. The electrochemical E-DNA antibody sensor readily supports multiplexed detection. Here, (left, perpendicular orientation) a microfabricated chip containing eighteen $500 \times 500 \mu\text{m}$ sensors, arranged in six three-pixel clusters, was employed. Each cluster is directed against a different antibody. Thus, the device supports the simultaneous, triplicate measurement of six different targets. Copyright 2012 American Chemical Society. Reprinted with permission from ref. 33, R. J. White, H. M. Kallewaard, W. Hsieh, A. S. Patterson, J. B. Kasehagen, K. J. Cash, *et al.* Wash-free, electrochemical platform for the quantitative, multiplexed detection of specific antibodies. *Anal. Chem.*, 2012, **84**(2), 1098–1103.

subject to challenges such as photobleaching, autofluorescence, and dissociation of organic dyes used in live cells.

Other methods

There are other promising methods of detection: for example, silver staining, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), confocal laser scanning microscopy, resonance light scattering, and radioimmunoassay. As can be seen in Table 5, silver staining and confocal laser scanning microscopy have the lowest limits of detection, in the pg to ng mL⁻¹ and pg mL⁻¹ ranges, respectively. They are also suitable for the detection of different analytes, such as viral nucleic acids, antigens, and antibodies to the virus.

Radioimmunoassay is another highly sensitive and inexpensive procedure, as can be seen in Table 5. The selectivity of this method is also very high, and the experimental procedure is

similar to that of a standard ELISA. However, special precautions must be taken during the experiment because radio-labeled reagents are used. Resonance light scattering is another valuable technique, whose convenience and sensitivity make it a potential diagnostic tool in health care.

SPR is mainly used to track the binding dynamics of biologically important molecules. It can also be used for the quantitative detection of analytes, as was demonstrated in ref. 8. This method has an advantage over other surface spectroscopy techniques because it does not need a vacuum, and is able to produce a linear dependence of resonant energy on the analyte concentration. SPR spectroscopy has potential for multiplexing, particularly when it is integrated with multi-channel microfluidic devices (Fig. 4).¹²⁶

SERS is a technique that allows for producing an enhancement in the order of millions and billions over standard Raman spectroscopy, which makes it useful for sensitive detection (in



ng mL⁻¹ range) of various analytes based on the characteristic Stokes shifts. This method requires a skilled operator and the use of a Raman spectrometer, which is now available as a portable, bench-size or even handheld device.¹²⁷ In comparison with fluorescence, Raman has great potential for multiplex detection of various analytes. Overall, this makes SERS a potential diagnostic test method. All abovementioned methods are listed in Table 5.

7. Post-COVID-19 trends and future perspectives

Comparing different methods, nucleic acid detection following amplification (real-time RT-PCR and LAMP) has higher sensitivity than ELISA, with a fg mL⁻¹ vs. pg mL⁻¹ average detection limit, respectively. The average limit of detection for real-time RT-PCR is 5 fg mL⁻¹ ($n = 7$), and 49 fg mL⁻¹ ($n = 6$) for the LAMP method. Having a detection limit higher than that for the ELISA method, the PCR-based tests are not free of drawbacks. Their disadvantages include the need for expensive equipment, trained personnel, and time. It takes several hours from the time of sample collection for a real-time RT-PCR test to produce results. The LAMP technique overcomes this limitation and enables a shorter (less than 1 hour) time until results, without the need for expensive equipment.

The limit of detection of electrochemical methods is comparable to the one for amplification-based methods of nucleic acid detection. The highest limit of detection is demonstrated by electrochemical methods with a capacitive immunosensor²⁸ for the detection of the HIV p24 antigen, which has a limit of detection of 7.9×10^{-5} fg mL⁻¹, and is accomplished in 20 minutes. The electrochemical methods are diverse in their principle, with some relying on capacitance measurements,²⁸ while others employ voltammetry⁴⁶ or amperometry.⁶⁰ They are also less restrictive in terms of the detected analyte, and are designed for the detection of viral particles, whether RNA⁴⁶ or protein²⁸, as well as antibodies (Fig. 5). The electrochemical methods are rapid and diverse. Some of them could be developed into low-cost point-of-care tests.

Amplification-based methods for the detection of nucleic acids, as well as ELISA-based methods for the detection of immune response in the form of antibodies, are two methods that are routinely used and demonstrate high sensitivity and specificity. However, they require time and proper equipment. The future of viral diagnostics lies in the point-of-care methods that can produce results within minutes, and do not require special equipment. The recent development of rapid diagnostics of SARS-CoV-2 in saliva¹³⁰ demonstrates the possibility of such a method. Other possible methods that can be used in point-of-care testing include portable antibodies/antigen test kits, fluorescence immunoassay devices, as well as portable RT-PCR and RT-LAMP devices.

Comparing the time it takes for different tests to produce results, lateral flow immunoassays offer a faster time to results, as compared to other methods (usually several minutes).

Electrochemical detection is another rapid diagnostics method, which takes less than 1 hour to produce results. Real-time RT-PCR takes the most time out of all test methods.

Enormous interest in virus detection on the wake of the COVID-19 pandemic is likely to reshape this area, directing efforts towards creating detection methods that have a fast time to results, high simplicity, high throughput, and are relatively low-cost. However, the specificity and selectivity of those tests should remain at least on par with well-established methods, such as PCR and ELISA.

Express testing for COVID-19 is necessary to contain the infection, and is particularly important for testing in airports, places of public gathering, transportation hubs and malls. During the pandemic, fast point-of-care tests on the scale of millions per day are required to minimize the mass quarantining of people, and to avert the harsh effects of lockdowns and travel bans on the economy and the wellbeing of people. Tests that take several days, such as the PCR test of the nasopharyngeal swabs, can result in the spread of infections since tested people will not know about their infection status until 5–7 days have passed. Today, there is a focus on developing express COVID-19 detection methods, especially using portable devices. There are several potential methods that provide test results within minutes or hours. One of them is the express RT-PCR test kit. These kits were developed by Cepheid ("Cepheid Xpert Xpress"), Roche ("Roche cobas")¹¹⁷ and Abbott ("ID NOW").¹³¹ These are already used in hospitals and ERs. According to published research, their accuracy matches that of the regular RT-PCR.¹¹⁷ This fact is a benefit of portable PCR devices, because with a sensitivity and specificity equal to that of stationary PCR, and with a significantly shorter time to results, they can be used for point-of-care testing. Some of these tests, such as "ID NOW", are approved by the FDA. Another express COVID-19 testing method is RT-LAMP. RT-LAMP uses DNA polymerase with the capability of separating double-stranded DNA, which eliminates the need for cycling temperature and simplifies the procedure. Portable and rapid testing devices using this method have also been developed, such as "Talis One" by Talis Biomedical.¹³¹ This device provides results in 30 minutes, a time comparable to that of portable RT-PCR devices. Published research on the detection of RNA from SARS-CoV-2 by RT-LAMP shows 100% agreement between the RT-LAMP and RT-PCR methods.¹¹⁸ Both RT-LAMP and RT-PCR can be used for the detection of viral RNA in the saliva from a person. Currently, the main method of detection is the same as with nasopharyngeal swabs – RT-PCR. Saliva was reported to be a suitable sample for COVID-19 testing by the University of Illinois. Researchers at the Yale School of Public Health and Hokkaido University even commented that PCR testing of saliva provides more accurate results than testing of nasopharyngeal swabs. Collecting saliva does not need medical staff and is more comfortable for a patient. This allows for more frequent testing without putting pressure on medical staff. Several procedures have been developed to pretreat saliva before PCR because saliva is more of a chemically complex matrix than nasopharyngeal swabs. Some of these procedures were proven to be successful, such as the protocols from the University of Illinois



and Fluidigm's "Advanta Dx SARS-CoV-2 RT-PCR".¹³⁰ If the current saliva testing methods obtain governmental permission, they can replace the testing of nasopharyngeal swabs. The detection of viral antigens in blood or nasopharyngeal swabs is also a promising method. Benefits include it being relatively cheap, fast, and portable. One such example is the "Sofia SARS Antigen Fluorescent Immunoassay" by Quidel, which was approved for use in healthcare organizations. Detection of antibodies to the virus is also available in portable devices as a rapid test (e.g., "COVID-19 IgG/IgM Rapid Test Cassette" by Zhejiang Orient Gene Biotech Co., Ltd.). The main benefits are the ease of use and speed (results are available in 10 minutes, and the only requirement is the addition of a drop of blood and the provided buffer on a test slide).¹²⁰ Antibody detection, however, is efficient only after several days have passed since the onset of symptoms. Both antibody and antigen tests are easier to use and cheaper, but they are less sensitive than RT-PCR. They can be used after the onset of symptoms to avoid a false negative during early testing. There are other rapid tests, such as pulse-controlled amplification (20 minutes),¹²³ reverse-transcription recombinase-aided amplification (5–15 minutes),¹²⁵ and other methods. However, these approaches are all new research studies requiring proficient scientists, and are confined to the laboratory, so they are not the main focus of today's trends.

Abbreviations

| | |
|-------------------|---|
| AFM | Atomic-force microscopy |
| Ab | Antibodies |
| CDC | Center for Disease Control |
| CMV | Cytomegalovirus |
| CoV | Coronavirus |
| CRISPR | Clustered regularly interspaced short palindromic repeats |
| cRNA | RNA copies |
| DNA | Deoxyribonucleic acid |
| ELD ₅₀ | 50% egg lethal dose |
| ELISA | Enzyme-linked immunosorbent assay |
| HA | Haemagglutinin |
| HAU | Hemagglutination unit |
| HAV | Hepatitis A virus |
| HCV | Hepatitis C virus |
| HDV | Hepatitis D virus |
| HEV | Hepatitis E virus |
| HFV | Hemorrhagic fever virus |
| HIV | Human immunodeficiency virus |
| LAMP | Loop-mediated isothermal amplification |
| LOD | Limit of detection |
| MERS | Middle east respiratory syndrome |
| NA | Neuraminidase |
| NP | Nucleoprotein |
| NS | Nonstructural |
| PCR | Polymerase chain reaction |
| PFU | Plaque-forming unit |
| RNA | Ribonucleic acid |
| RSD | Relative standard deviation |

| | |
|--------------------|---|
| RT-PCR | Reverse transcription polymerase chain reaction |
| Rxn | Reaction |
| SARS | Severe acute respiratory syndrome |
| SERS | Surface-enhanced Raman spectroscopy |
| SPR | Surface-plasmon resonance |
| TCID ₅₀ | 50% tissue culture infective dose |
| WHO | World Health Organization |

Funding statement

This research is supported by the Nazarbayev University (NU) Faculty Development Competitive Research Grant no. 090118FD5352 (Kazakhstan), and the NU Faculty Development Competitive Research Grant no. 110119FD4517 (Kazakhstan).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to acknowledge Yunona Bukasova for proofreading the paper.

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